EFFECT OF NITROGEN FERTILIZATION AND FORAGE MATURITY ON THE NUTRITIVE VALUE OF BAHIAGRASS

A Senior Scholars Thesis

by

NICOLE MARION KENNEY

Submitted to the Office of Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

April 2010

Major: Animal Science

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	Jason E. Sawyer
Associate Dean for Undergraduate Research:	Robert C. Webb

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ABSTRACT

Effect of Nitrogen Fertilization and Forage Maturity on the Nutritive Value of Bahiagrass. (April 2010)

Nicole Marion Kenney
Department of Animal Science
Texas A&M University

Co-Advisors: Dr. Tryon A. Wickersham
Dr. Jason E. Sawyer
Department of Animal Science

Bahiagrass (*paspalum notatum*), a forage resource in the southern United States, often has lower forage quality than other forages, but may require fewer nutrient inputs. Our objectives were to determine the effects of N fertilization and maturity on nutritive value and yield of bahiagrass. Treatments were arranged as a 4 × 4 factorial with 4 levels of N fertilization (0, 45, 90, and 135 kg N/ha) and 4 maturities (3, 5, 7, and 9 wk after N fertilization). An established stand of Bahiagrass located in Brazos County, TX, was divided into 3 blocks with all treatment combinations contained in each block. Increasing provision of N tended ($P = 0.06$) to quadratically increase in DM yield (3354, 4386, 4876, and 5182 kg DM/ha for 0, 45, 90, and 135 kg N, respectively). Advancing maturity increased DM yield quadratically ($P < 0.01$; 3206, 4580, 4894, and 5119 kg DM/ha for 3, 5, 7, and 9 wk, respectively). A maturity × N interaction ($P = 0.02$) was observed for forage CP concentration. Increasing N resulted in more rapid declines in CP with advancing maturity. At 3 wk CP was 8.0% for 0 N and 11.6% for 135 N. At 9 wk the CP was 5.0% for 0 N and 6.6% for 135 N. In situ OM digestibilities were

determined on samples from wk 5, 7, and 9. A maturity by N interaction (P = 0.03) was observed for the rapidly degraded (A) fraction of OM. At 5 wk maturity the A fraction decreased with increasing N, whereas at 9 wk maturity the A fraction increased with increasing N. The B fraction was linearly reduced (P < 0.01) and the C fraction was linearly increased (P < 0.01) with advancing maturity. At a fixed passage rate of 3%/h, the calculated extent6 of OM degradation was 58.6, 54.9, and 53.4% for maturities 5, 7, and 9, respectively (linear, P < 0.01). Overall, additional fertility increased bahiagrass CP content, despite more rapid declines with advancing maturity, and maturity was the primary driver of bahiagrass degradability.

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CHAPTER I

INTRODUCTION

Bahiagrass

In the southern United States, Texas particularly, growing conditions dictate that warm-season grasses are the dominate forage source. Consequently, bahiagrass (*Paspalum notatum)*, a warm-season perennial grass, is commonly grown for use as both hay and pasture. Bahiagrass is native to South America and was introduced to the United States in the early 1900s. Currently, bahiagrass covers a great deal of acreage all across the Southern United States.

A challenge in using bahiagrass is that warm-season forages typically display lower forage quality which negatively impacts their feeding value to livestock and, ultimately, compromises livestock performance (Skerman and Riveros, 1990). Warm-season perennial grasses have been found to be of lower nutritive value when compared to cool season grasses and legumes, with warm-season grasses having higher fiber and lower crude protein contents, comparatively (Ogden et al., 2006). Reduced forage quality is, in part, due to the increased metabolic efficiency of warm-season grasses which in turn results in a lower proportion of nutrients being available to the animal (Johnson et al., 2001). While forage quality is often lower for bahiagrass species than cool season forages, management and environment are key factors in determining the value of

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produced forages. Understanding these factors has the potential to improve forage quality and system productivity.

Despite the negative attributes of warm-season forages, production conditions demand their inclusion in pastures. Bahiagrass is particularly attractive as a forage resource because of its ability to survive on sandy, infertile soils and to recover from heavy grazing with only moderate fertilization. Furthermore, bahiagrass characteristically makes thick, tight sods which allow it to be tolerant of continuous grazing while effectively competing with weeds. When compared with cool season forages, warm-season forages generally produce a more DM yield (Skerman and Riveros, 1990).

The objectives of this review are to 1) describe the impact of nutrient provision, more specifically nitrogen (**N**) fertilization, on bahiagrass quantity and quality and 2) quantify the impact of maturity on forage quantity and quality.

Nitrogen fertilization

Fertilization of grasses for use as pasture or hay typically results in increases in forage quality and yield. As a result, N fertilization is commonly practiced by producers in the southern U.S. and beyond. Producers have a variety of options as to type of fertilizer and whether the nutrients are immediately available or slowly released. Urea and ammonium nitrate are two fertilizers that are commonly used; both are sold by a variety of vendors commercially.

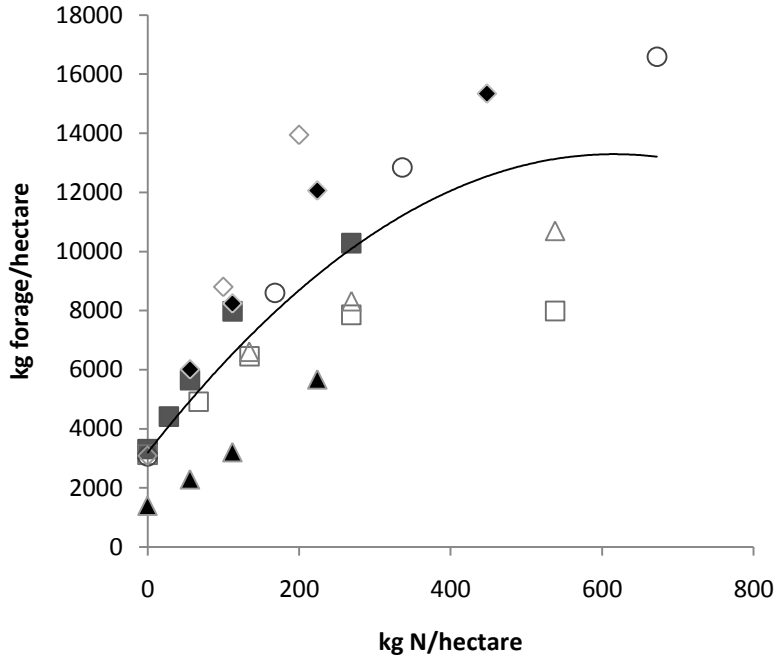

Figure 1. Effect of N fertilization on bahiagrass yield (Engibous et al. 1958 □; Beaty et al. 1960 ■; Evans et al. 1961○; Beaty et al. 1963▲; Ruelke and Prine 1971 Δ; Blue 1988 ◊; Burton et al. 1997 ♦)

Increasing levels of N fertilization have resulted in greater bahiagrass yield across a number of studies and sites (Engibous et al., 1958; Beat et al., 1960; Evans et al., 1961; Beaty et al., 1963; Ruelke and Prine, 1971; Blue, 1988; Burton et al., 1997; Figure 1). The response to N, across the multiple studies reported here, appears to be quadratic. Indicating that with each incremental increase in N fertilizer the yield response is smaller than the one preceding it. Eventually the response plateaus and no further increases in yield are observed with additional N. This plateau or break point is generally observed at 600 kg N per hectare, but varies between studies and is dependent on other factors such as rainfall. Other studies, for example Engibous et al. (1958) and Ruelke and Prine (1971) indicate that a plateau is reached at a lower level of N fertilization. Engibous et

al. (1958) found that optimum fertilization was at 269 kg N per hectare applied in two applications per year and that single applications of N over 134 kg N per hectare depressed yield. Prine and Burton (1956) found similar results, with Bermudagrass, and recommended 267 kg N per hectare for maximum production. Time of N application and the number of applications has been reported to havce very little effect on annual forage yields or quantity of N absorbed by the root system (Blue, 1988). While it has been reported that that time and number of N applications does not influence yield of N absorption it is generally recommended that fertilization at high levels be done in several applications to avoid environmental impacts such as N run off.

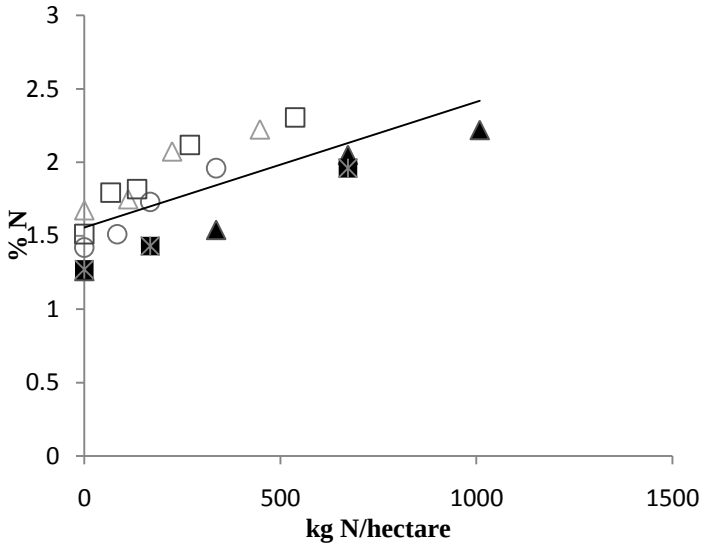

Figure 2. Effect of N fertilization on N content (Engibous et al. 1958□; Evans et al. 1961■; Beaty et al. 1963○; Ashley et al. 1965▲; Mathias et al. 1978Δ)

Nitrogen fertilization of bahiagrass linearly increases the N concentration of Bahiagrass (Engibous et al., 1958; Evans et al., 1961; Beaty et al., 1980; Ashley et al., 1965; Mathias et al., 1978; Figure 2). The greatest amount of total N occurred at the highest N fertilization levels (Johnson et al., 2001). Puoli et al. (1991) found similar results when providing N fertilization to two native warm-season grasses, switchgrass and big bluestem. Increasing levels of N fertilization increase protein fractions A, B and C, which are non protein nitrogen, degradable true protein and undegrable protein respectively (Johnson et al., 2001). Additionally, greater accumulation of nitrate, a portion of fraction A, has been observed in response to higher levels of N fertilization (Johnson et al., 2001). Cuomo and Anderson (1996) found that increased fertilization of switchgrass, big bluesteam and indiangrass led to an average increase in CP by 18%,

with 65% of that being ruminally degradable protein, fraction B, and the remaining 35% being undegradable, fraction C.

Limited supplies of ruminally degradable N limits the pool of ruminal N available for microbial synthesis and reduces digestibility (Brown and Pitman, 1991). Increased N levels allow for a greater proportion of the degradable intake protein requirement to be met, which allows for greater synthesis of microbial crude protein, thus a greater proportion of the animal's requirements are met. A common limiting factor of digestibility in forages is percentage crude protein, or more specifically the percentage of ruminally available N in forages. Feeding a forage with low N depresses both intake and forage fermentation within the rumen (Koster et al., 1996). Without an adequate N supply rumen microbes are unable to work to full capacity, which ultimately results in a failure to meet the amino acid and energy requirements of the animal. This in turn can lead to compromised performance, which is measured in terms of average daily gain, pounds of milk produced or the weaning weights of calves. Therefore, increases in forage crude protein content have the potential to improve livestock performance and profitability. However, increases in N fertilization have been found to increase passage rate (Puoli et al., 1991). An increase in passage rate competes with the increased digestibility afforded by the increased forage quality provided by N fertilization.

Two studies, Engous et al. (1958) and Evans (1961), provided sufficient data for calculating N per hectare (Figure 3). Engibous et al. (1958) found the maximum

response to N fertilization, this is evident in that the line of best fit for their data is quadratic, while Evans (1961) found a linear response, indicating that the maximum response had not been reached. This linear response is likely explained by greater water availability in Evans (1961) study, as the bahiagrass was irrigated, allowing it to utilize the applied N more effectively.

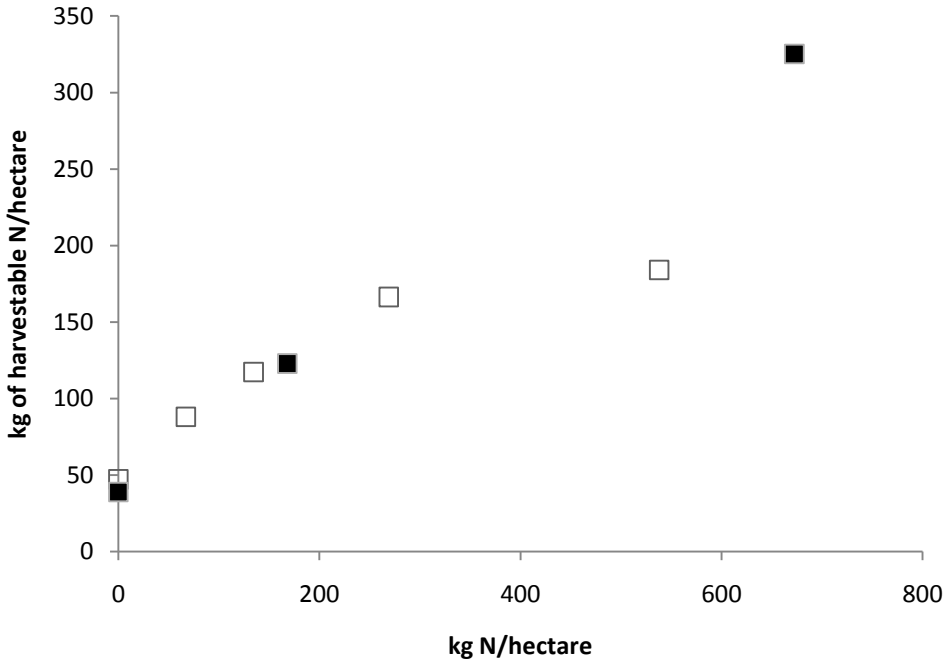

Figure 3. Effect of N fertilization on harvestable kg N per hectare (Engibous et al. 1958 □; Evans et al. 1961■)

A positive correlation has been reported between increasing levels of N fertilization and forage quality (Ogden et al., 2006; Johnson et al., 2001; Puoli et al., 1991). Increased N fertilization results in decreased NDF in bahiagrass (Johnson et al., 2001). In theory, a decrease in NDF, which consists of hemicellulose, cellulose and lignin, results in greater

digestibility because a larger proportion of carbohydrate is available as highly soluble non-structural carbohydrates (Van Soest, 1994). In contrast to this, Johnson et al. (2001) found that in vitro organic matter digestibility (**IVOMD**) of grass was not affected by increasing levels of N fertilization. This is further supported by the findings of Burton et al. (1997) who found that in vitro dry matter digestibility (**IVDMD**) of grass was not affected by N fertilization. Stanley et al (1977) found that N had no effect on cell wall constituents. This is significant because when cell wall constituents rise over 50 to 60 percent voluntary intake is depressed (Van Soest, 1965). The limited impact of N fertilization on digestibility may be explained in part because increasing levels of N fertilization has little to no effect on the concentration of ADF, which consists of cellulose and lignin, which is the least digestible fraction. Additionally, the effects of N fertilization on DM digestibility are inconsistent, with some studies observing increases while others report depressions as high as 3% (Skerman and Riveros, 1990).

Threshold of effectiveness

Economic and environmental sustainability of forage production and utilization is dependent, in part, on determining the optimum level of N fertilization. Threshold of effectiveness for N fertilization is the point at which any additional N fertilization does not translate into an equivalent increase in forage and, ultimately, animal performance. Lower N absorption efficiencies translate into decreased profitability of forage and animal production systems (Blue, 1988). Beaty et al. (1980) found that the efficiency of N use by bahiagrass decreased as the rate of application increased. Increasing N

fertilization from 84 to 168 kg N per ha resulted in a reduction in dry forage yield increases per kg of applied N form from 54.6 to 5.2 kg per kg of applied N (Beaty et al. 1980). Johnson et al. (2001) found that the initial application of N doubled bahiagrass production, but that no further increases were seen with additional applications. Even if some effectiveness is seen in applying N late in the growing season the increases in production may not be enough to offset the costs of further fertilization (Johnson et al., 2001).

Regular use of high levels of N becomes a problem because as the amount of N applied rises the amount of N recovered in the forage decreases (Ashley et al., 1965). Blue (1988) found that average N recovery of bahiagrass was 80 to 85 % at 100 and 200 kg N per hectare. The potential for lost N to contamination of ground water, due to repeated applications of high levels of fertilizer is a concern. Additionally, increased N in animal waste has the potential to negatively impact environmental quality. One solution may be the use of infrequent fertilization, where forages are produced on land that is not fertilized every year. Used as a management technique infrequent fertilization has the potential to increase N recovery (Ashley et al., 1965). However, this system sacrifices forage yield and quality in years when fertilizer is not applied.

Forage maturity

Forage maturity has been found to be largely responsible for declining forage quality (Newman et al., 2009). Increased maturity in grasses results in thickening of the

secondary layer with concomitant lignification, or more simply put an increase in the proportion of lignin (Van Soest, 1994). Lignin, a polymer of phenylpropanoids, serves to increase the strength and rigidity of plants while also decreasing water loss. As a result of the interaction between lignin and structural carbohydrates, hemicellulose, cellulose and pectin, increasing lignin acts a barrier to fiber degradation by rumen microbes (Newman et al., 2009). As plants mature structural carbohydrates such as cellulose and hemicellulose also increase. Young plant cells, in comparison, have a greater portion of cell contents when compared to more mature cells. Young plant cells are more digestible and more easily ruminated, but by weight are consumed in the same quantity as more mature cells (Van Soest, 1994). Consequently, feeding forages lower in structural carbohydrates and lignin will ultimately result in greater digestibility. Lignin content is especially important as lignin is the most important single fiber component limiting nutrient availability (Van Soest, 1994).

Generally, forage from first cuttings are lowest in cell wall constituents (Stanley et al., 1977). Arthington and Brown (2005) found that increased maturity of bahiagrass, 10 wk regrowth versus 4 wk, resulted in a 38% decrease in CP and an increase in ADF of 7% and in acid detergent lignin of 3%. However, IVOMD was minimally decreased, 1.1%, when maturing from 4 to 10 wk and no decrease in apparent digestibility were observed (Arthington and Brown, 2005). A summary of the data indicates that increasing maturity results in decreased CP (Figure 4).

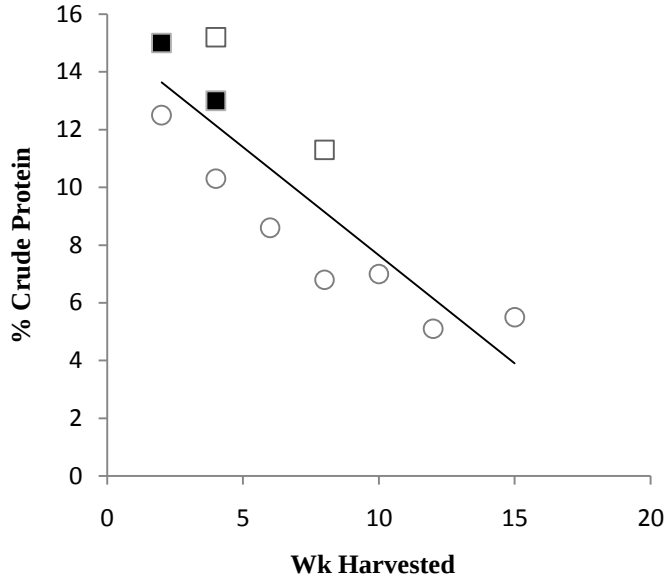

Figure 4. Effect of maturity on crude protein (Utley et al. 1974 □; Brown et al. 1976 ■; Brown and Mislevy 1988 ○)

Clipping frequency has been found to affect forage quality. More frequent clipping can result in higher quality forages. Stanley et al (1977) observed that cell expansion and cell wall constituents are completed in less than 30 days. Newman et al. (2009) report that forage digestibility and CP content declined sharply after 35 to 40 days in warm-season grasses.

Environmental influences

Preharvest environmental factors, available water and temperature, during the growing season affects the quality and yield of forage produced, though due to the myriad of variables involved in a field study it is difficult to attribute variations to specific

conditions. These environmental factors have a marked influence on the distribution of cell wall contents (Van Soest et al., 1978).

Moisture

In many cases, especially in parts of Texas, water availability often limits bahiagrass growth. Henderson and Robinson (1982b) reported that during a wet year (100 cm from April 1 to November 1) twice as much bahiagrass was produced when compared to drought year (35 cm rainfall) at the same location. Irrigation during times of low rainfall will increase the yields of fertilized bahiagrass (Ashley et al. 1956) allowing forage to demonstrate the potential yield increases which are enabled by N fertilization. However, irrigation has been found to result in more rapid stand deterioration (Ashley et al.1965).

In addition to enabling forage to meet its growth potential irrigation has the potential to increase N recovery. Ashley et al (1965) found that irrigation increased N recovery of bahiagrass from 57 percent in unirrigated plots to 88 percent in irrigated plots. Irrigation increases N recovery because of it produces higher yields allowing a greater percentage of N to be utilized. In the event that water requirements are not met forage quality has been found to be negatively affected. Henderson and Robinson (1982a) found that water stress reduced the DM digestibility of bahiagrass, though it did not significantly change cell hemicullulose and lignin. In contrast, it did increase ADF which indicated cellulose concentrations increased (Henderson and Robinson 1982a).

Temperature

Johnson et al. (2001) found that peak production of bahiagrass occurred during late June and early July, which coincides with findings by Prine and Burton (1956) regarding switchgrass and big bluestem. Gates et al (2001) determined that 86% ofbBahiagrass production occurs during the 6 warmest months of the year. However, during these times of high production the lowest IVOMD values are observed (Johnson et al., 2001). These two observations are the result of metabolic efficiency in response to high temperatures, which decreases the leaf to stem ratio, and produces a greater ADF concentration (Johnson et al., 2001). Cell wall material deposited at a higher temperature is more lignified, concomitantly, less storage carbohydrates accumulate in leaves, both factors resulting in lower digestibility (Nelson and Moser, 1994). This is further supported by research that has shown that ADF concentrations are more sensitive to the environment than NDF concentrations (Henderson and Robinson, 1982a). In addition to effecting fiber content, high temperatures have been shown to result in a depression of CP (Johnson et al., 2001). In short, there is an inverse relationship between temperature and forage quality (Henderson and Robinson, 1982a).

Following maturity, temperature is the second most influential factor on the nutritive value of grasses (Van Soest et al., 1978). Increasing light intensity has been found to increase soluble carbohydrate content of grass which in turn increases digestibility (Van Soest et al., 1978). However, during the summer months this process is in direct competition with the decreased forage quality caused by rising temperatures.

Consequently, the advantage gained by a greater length of day during the summer months is offset by the high temperatures recorded in the Southern U.S. during this period.

Measures of nutritive value

Nutritive value is measured in a variety of ways. Typically, our goal is to determine the availability of nutrients, which are generally expressed in terms of total digestible nutrients (**TDN**), a measure of energy, and CP. These two measures of nutritive value together serve as indicators of energy and protein supply and support balance between energy and protein. Crude protein can be split into three fractions, non protein nitrogen (A), degradable intake protein (B), which is available to the rumen microbes, and undegradable intake protein (C), which is not available to microbes and only undergoes enzymatic digestion. Balancing a ration to achieve rumen microbe efficiency is necessary to maximize animal production, whether that is average daily gain, reproductive efficiency or milk production. In order to do this, degradable intake protein must be fed at 13% of TDN consumed.

In addition to TDN and CP, nutritive value can also be measured by the detergent fiber system. The detergent fiber system measures nutritive value by determining the portion of cell wall content and degree of lignification. The detergent fiber system, though having problems of its own, replaced the crude fiber system. The crude fiber system solubilizes a portion of hemicellulose resulting in a failure to produce a clean measure of fiber while also underreporting percentage fiber. The detergent fiber system consists of

two analyses, NDF and ADF. Neutral detergent fiber solubilizes cell contents, thus giving a measure of hemicellulose, cellulose and lignin. Often NDF is used as a predictor of forage intake. Acid detergent fiber goes one step further and solubilizes hemicellulose, resulting in a measure of lignin and cellulose. Acid detergent fiber is often used as a predictor of forage digestibility.

In situ

In situ trials are used to determine the ruminal disappearance of a nutrient. Replicates of a forage or grain sample are incubated in the rumen over time, generally ranging from 0 to 72 hours, to measure rate and extent of digestion. Proximate analyses of the sample residues combined with information regarding passage rate allow for a determination of the actual availability of nutrients. Nutrient availability is limited by the extent of unavailable matter and competition between rates of passage and digestion, with decreases in digestion as passage rates increase (Van Soest, 1994). For example, the ruminal degradation rate of potentially degraded N in warm-season grasses is 6-18% per hour, whereas in legumes it is 24-44% per hour, with legumes being faster because of the lower concentration of hemicellulose (Brown and Pitman, 1991). As a result, at the same rate of passage grasses are higher in undegraded intake protein. Crude protein is split into three fractions in order to account for the different rates at which the fractions are degraded within the rumen. Fraction A, known as NPN, is already degraded at 0 time, Fraction B is the potentially degradable portion and Fraction C is undegradable (Broderick, 1994). Unlike fibrous portions, there is no lag time associated with N

disappearance (Brown and Pitman, 1991). In situ trials enable one to know how much degradation of N occurs within the rumen for specific durations.

Inconsistency in degradability studies can be attributed to animal characteristic, substrate characteristic and procedural aspects such as rinsing techniques (Vanzant et al., 1998). The interactions between the various factors make the standardization of technique across all feedstuffs nearly impossible; however through repeated trials the techniques have been refined. It is generally accepted that the bottom of the rumen is the best site of incubation and that a larger bag to sample size ratio minimizes error (Van Soest, 1994). The interaction between bag pore size and the grind size used for samples and its effect on the disappearance of feed constituents have been largely standardized. The majority of literature reports use of in situ bags with a pore size ranging from 40 to 60 μm and a grind size of 2mm for grinding (Vanzant et al., 1998).

Diet composition has a major influence on the degradation of substrates. Anything that would significantly change the microbial population of the rumen, for example the use of a high starch diet while incubating forage samples, has the potential to influence results. Consequently, researchers generally feed a diet identical or very similar to that of the feed substrate they are incubating. Additionally, the frequency of feeding effects the dinural fluctuations in bacterial and protozoal populations which can skew degradation results on samples with shorter incubation times (Vanzant et al., 1998). Vanzant et al (1998) reported that feeding more frequently maintains a more constant

rumen environment. Furthermore, Meyer and Mackie (1986) reported that bacteria entered synthetic bags more rapidly and in great numbers when feeding was more frequent which allows for greater fermentation.

Yet, even with these refinements there are still errors which need to be corrected. With longer incubation times contamination with microbial protein confounds results (Broderick, 1994). However, this can be corrected for using the methods described by Mathis et al. (2001). Additionally, in situ procedures result in the overestimation of Fraction A, the underestimation of the rate at which Fraction B is degraded and overestimation of Fraction C (Broderick, 1994). Nonetheless, fraction estimation errors tend to cancel each other out resulting in biologically reliable values (Broderick, 1994).

Finally, there has been a great deal of variation in rinsing procedures following ruminal incubation (Vanzant et al., 1998). Procedures range from hand rinsing to mechanical rinsing with a standard washing machine. Furthermore, the decision of end points among hard rinsing techniques varies with both time and clarity of water being used as the deciding factor. Currently, there appears to be no singular best method regarding rinsing procedures.

Conclusion

Ultimately, as a result of the broad use of bahiagrass as forage produced for consumption by cattle it is important to understand the effects of maturity and fertilization, as well as

the contributing environmental effects, on forage quality. Equally as important is to strive to find the best combination of the two in order to produce forage of the highest quality while still being economical for the producer. With the increased reliability of in situ results, the technology is now readily available to provide this information.

CHAPTER II

METHODS

Collection of experimental forages

The experimental protocol was approved by the Institutional Animal Care and Use Committee at Texas A&M University.

This study evaluated the effects of N fertilization and maturity on the in situ degradability of bahiagrass (*Paspalum notatum*) DM, OM, and crude protein. Treatments were arranged as 4 × 4 factorial with the first factor consisting of 4 levels of N fertilization and the second factor representing 4 maturities. An established stand of bahiagrass located in Brazos County, TX, was divided into 3 blocks each block was subsequently divided into 4 3.0 × 3.7 m plots, according to soil test. All plots were fertilized with 61.5 kg of P per ha and 78.5 kg of K per ha. Within each block plots were assigned to one of levels of nitrogen fertilization (0, 44.8, 89.7, or 134.5 kg N per ha. P and K was applied on May 13th and N was applied on May 14th, 2009.Clippings were collected at two weeks intervals with the first and final collections occurring on June 4, July 16, 2009, respectively, using hand shears. Such that samples were collected 3, 5, 7, and 9 wk after fertilization. At each collection 5 samples were collected from each plot using a sampling square (25.4 × 30.5-cm), to determine average forage production per hectare. Subsequent to collection samples were weighed and placed in a forced-air oven at 60°C for 96 hours. Due to a severe drought during the summer of 2009 supplemental water, 2.54 cm , was provided to each plot during wk 4 and 6.

Laboratory analyses

Samples were initially ground through a Wiley Mill to pass a 2-mm screen. Samples were composited for each sampling time within plot. A portion of each sample was retained for use in the *in situ* trial. The remaining sample was ground through a Wiley Mill to pass a 1-mm screen. Dry matter was determined by drying samples at 105°C for 24 h, and OM was determined as loss in dry weight upon combustion for 8 h at 450°C in a muffle furnace. Nitrogen content was determined by (Elementar Nitrogen Analyzer, Elementar Mt. Laurel, NJ). Crude protein was calculated as N × 6.25. All samples were analyzed for NDF and ADF sequentially with a fiber analyzer (model 200, Ankom Technology, Fairport, NY), with sodium sulfite and amylase omitted and without correction for residual ash.

In situ procedures

Three ruminally cannulated steers (240 kg initial BW) were used to determine in situ disappearance kinetics of bahiagrass DM and OM. Steers were housed in an enclosed, climate controlled barn in individual pens (2.1 × 1.5-m) with continuous lighting and ad libitum access to water and a trace mineral block (97% NaCl, Min 1.80 % Ca, 1% S, 3000 ppm Mn, 2500 ppm Zn, 1500 ppm Fe, 150 ppm Cu, 90 ppm I, 25 ppm Co, 10 ppm Se, United Salt Corporation, Houston, TX). Steers will also provided ad libitum access to Bermudagrass hay were fed 454g of cottonseed meal two times per day to ensure ruminal nitrogen was not limiting.

Five grams of bahiagrass was sealed in Dacron bags (10 × 20 cm; average pore size 50 ±10-µm; Ankom, Fairport, NY). Due to insufficient sample in situ analysis was only preformed on samples collected 5, 7, and 9 wks after fertilization. Before insertion in the rumen samples were incubated in tepid water (approximately 39°C) for 20 minutes to decrease the lag time associated with wetting within the rumen. Within the rumen samples were contained in a 60 × 25 cm mesh laundry bag which was weighted. Samples were inserted in the rumen in reverse order, with the 72 hr sample being inserted first, allowing all samples to be removed and undergo the same rinsing procedure. Incubation times were 2, 6, 12, 24, 48 and 72 hours. A 0 hour sample was included and underwent the same preincubation and rinsing procedures as the other samples. Following removal samples were rinsed in a top loading washing machine set at a low water level. Samples were subjected to ten cold water rinses, with each cycles consisting of one minute agitation and two minutes of spin cycle. Following the rinsing cycle samples were dried to a constant weight at 60°C in a forced-air oven. Upon removal from the oven samples were allowed to air equilibrate and dry sample weights were recorded. Post incubation the samples were analyzed for DM and OM using the methods outlined above. These values were used to determine DM and OM disappearance within the rumen.

Organic matter residues remaining after incubation were used to fractionate OM into three fractions: A immediately soluble; B potentially degradable; and C complete undegradable. The A fraction is measured as the OM that washes out of the bag when

rinsed with water (i.e., the OM that is lost from the 0 h bag). The C fraction is simply the OM remaining in the bag incubated for the longest duration (72 h). The B fraction is calculated as 100% - (A – C). To calculate the degradability of the B fraction the proportion of the OM classified as B is multiplied by $k_d/(k_d+k_p)$. In this equation k_d represents the rate of OM degradation, measured as the slope of the regression of the natural logarithm of OM remaining against time. Similarly, k_p represents the rate of passage out of the rumen and was be determined using existing in vivo measures and estimates of ruminal rates of passage.

Statistics

Forage nutritive value data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). Model terms were maturity, N fertilization, and the maturity by N fertilization interaction. The random term in the model was block. Treatment means were determined using the LSMEANS procedure of SAS. Orthogonal polynomial contrasts (linear and quadratic) were used to partition treatment sums of squares. In situ disappearance variables were analyzed using the MIXED procedure of SAS. Terms in the model were maturity, N fertilization, and the maturity by N fertilization interaction with block and steer included as random terms. Treatment means were determined using the LSMEANS procedure of SAS. Orthogonal polynomial contrasts (linear and quadratic for N fertilization and linear for maturity) were used to partition treatment sums of squares.

CHAPTER III

RESULTS AND DISCUSSION

Nutritive value of bahiagrass

Rainfall for May, June, and July (through study completion, July 16, 2009) was 3.6, 0, and 0 cm. Which is in contrast to the averages of 12.8, 9.6, and 4.9 cm for those months, respectively. In an attempt to mimic more normal rainfall patterns 2.54 of water was applied on the 8th and 25th of June. However, this supplemental provision of water did not bring total moisture close to normal and water availability likely influenced our observations.

There were no significant maturity × N interactions for either DM of N yield ($P > 0.62$). However, increasing provision of N tended ($P = 0.06$) to increase DM yield quadratically; Table 1. This response is consistent with the findings of Engibous et al., (1958), Beaty et al., (1960), and Burton et al., (1997). Engibous et al. (1958) reported an increase in DM yield from 3124 to 6451 kg per ha when increasing N fertilization from 0 to 135 kg N per ha. Similarly, Beaty et al. (1960) reported an average increase, across three years, of 4658 kg DM per ha between treatments of 0 and 112 kg N per ha. Burton et al. (1997) reported 6010 kg DM per ha with a fertilization level of 56 kg N per ha and 8240 kg DM per ha when increasing N fertilization to 112 kg N per ha. Greater bahiagrass yield responses observed, using similar N fertilization treatments, by these authors, in comparison to the current project, are likely explained, at least in part, by greater water availability. While Engibous et al. (1958) and Burton et al. (1997) did not

report rainfall, Beaty et al. (1960) reported average, spanning the years of 1955 through 1957, rainfalls of 8.1, 11.4, and 16 cm for the months of May, June, and July, respectively.

Table 1. Effect of N fertilization and maturity on dry matter, N yield, and nutritive value of bahiagrass

N Fertilization Level[a]	Harvest Date	kg DM/ha	kg CP/ha	kg N/ha	CP%	OM%	ND%
0	June 4	2492	200.7	32.1	8.0	92.5	69.7
45	June 4	2996	272.5	43.6	9.1	93.1	71.1
90	June 4	3339	363.4	58.1	10.9	93.2	68.8
135	June 4	4000	463.6	74.2	11.6	93.2	70.6
0	June 18	3112	206.0	33.0	6.6	92.9	69.7
45	June 18	4650	335.5	53.7	7.2	93.2	71.3
90	June 18	5082	440.0	70.3	8.7	93.3	70.5
135	June 18	5476	499.3	79.9	9.2	92.5	70.2
0	July 2	3908	220.7	35.3	5.6	93.1	69.0
45	July 2	4781	290.0	46.4	6.0	93.6	69.8
90	July 2	5424	381.2	61.0	7.0	93.7	70.8
135	July 2	5464	412.0	65.9	7.6	93.8	69.4
0	July 16	3905	198.0	31.7	5.0	93.2	69.2
45	July 16	5118	262.4	42.0	5.1	93.9	69.2
90	July 16	5662	344.9	55.2	6.1	94.2	69.0
135	July 16	5792	383.2	61.3	6.7	93.6	68.4
SEM		379.7	0.54	4.5	0.63	0.26	0.63
Maturity		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Nitrogen		<0.01	<0.01	<0.01	<0.01	<0.01	0.1
Maturity × N		0.91	0.62	0.62	0.02	0.31	0.07
Linear							
Maturity		<0.01	<0.05	0.05	<0.01	<0.01	<0.01
Quadratic							
Maturity		<0.01	0.01	0.01	<0.01	0.37	0.03
Linear N		<0.01	<0.01	<0.01	<0.01	0.02	0.88
Quadratic N		0.06	0.35	0.35	0.82	<0.01	0.05

[a]kg N/ha

Advancing maturity from 3 to 9 wks increased DM yield quadratically ($P < 0.01$). This contradicts with the observations reported by Brown et al. (1976), who found that though

bahiagrass yield increased from May to June, which is likely attributed to depressed yields during May due to late establishment of the bahiagrass stand, yield decreased linearly over the next four months. As expected, increasing N fertilization level increased CP linearly ($P < 0.01$). Engibous et al. (1958) reported an increase in CP in bahiagrass from 9.4 to 11.3% when increasing from 0 to 135 kg N per ha. Mathias et al. (1978) reported similar findings regarding Midland Bermudagrass. It is likely that bahiagrass CP concentrations were lower for this trial, increasing from 6.3 to 8.7% when increasing N treatment from 0 to 135 kg N per ha, due to the effect of high temperatures during the trial which have been shown to depress CP in Bermudagrass (Johnson et al., 2001). Temperatures recorded in College Station, Texas for 2009 were a monthly average of 78, 86, and 89, with maximum temperatures recorded as 93, 106, and 105, for the months of May, June, and July, respectively. With increased maturity a quadratic decrease ($P < 0.01$) in CP was observed. This differs from previous findings, which report a linear decrease in CP with advancing maturity (Utley et al. 1974; Brown et al. 1976; Brown and Mislevy 1988). A maturity by N interaction ($P = 0.02$) was observed for CP concentration. Increasing N resulted in more rapid declines in CP with advancing maturity. Level of fertilization resulted in a linear increase ($P < 0.01$) on kg CP per ha. Advancing maturity tended to have a quadratic effect ($P = 0.01$) on kg CP per ha. These same effects, as a result of increased N fertilization and advancing maturity, are observed in kg N per ha.

There was a maturity × N interaction for CP content ($P = 0.02$) driven by an increase in the rate of CP decline with advancing maturity as N fertilization was increased. Samples collected on June 4th ranged from 8.0 to 11.6% CP for 0 and 135 kg N per ha, respectively, with 45 and 90 having intermediate value of 9.1 and 10.9 %. In contrast, samples collected on July 16th ranged from 5.0 to 6.7 for 0 and 135 kg N per ha, respectively. Over the course of the maturities measure CP concentrations decreased by 3.0, 4.0, 4.8, 4.9 % for 0, 45, 90, and 135 kg N per ha. Across maturities, increasing N fertilization resulted in a linear ($P < 0.01$) increase in CP content. Similarly, Engibous et al. (1958) reported an increase in CP in bahiagrass from 9.4 to 11.3% when increasing from 0 to 135 kg N per ha. It is likely that bahiagrass CP concentrations were lower for this trial, increasing from 6.3 to 8.7% when increasing N treatment from 0 to 135 kg N per ha, due to the effect of high temperatures during the trial which have been shown to depress CP in Bermudagrass (Johnson et al. 2001). Temperatures recorded in College Station, Texas for 2009 were a month average of 25, 30, and 32 °C, with maximum temperatures recorded as 34, 41, and 40 °C, for the months of May, June, and July, respectively. With increasing maturity a quadratic decrease ($P < 0.01$) in CP was observed. This differs from previous findings, which report a linear decrease in CP with advancing maturity (Utley et al. 1974; Brown et al. 1976; Brown and Mislevy 1988). Level of fertilization resulted in a linear increase ($P < 0.01$) on kg CP per ha. Advancing maturity tended to have a quadratic effect ($P = 0.01$) on kg CP per ha. These same effects, as a result of increased N fertilization and advancing maturity, are observed in kg N per ha.

A quadratic response ($P < 0.01$) to increasing N fertilization was observed on OM concentration. Harvest date had a linear effect ($P < 0.01$) on forage OM. Nitrogen fertilization had little effect on NDF ($P = 0.1$). This supports the finding of Cuomo and Anderson (1996), who observed that N fertilization had no effect on NDF concentration in warm season grasses. Advancing maturity resulted in a linear ($P < 0.01$) decrease in NDF. Johnson et al. (2001) observed a linear ($P < 0.01$) decrease in bahiagrass NDF concentration with increased levels of N, while increased maturity resulted in a linear increase ($P < 0.01$) of 2.8% in NDF concentration. Henderson and Robinson (1982b) reported a negative relationship between NDF and temperature. The linear decrease in NDF observed with advancing maturity can certainly be attributed to the increasing temperatures across harvest dates. With increasing levels of N fertilization a quadratic response ($P < 0.01$) is observed in ADF concentrations. This differs from the observation of Johnson et al. (2001) and Cuomo and Anderson (1996) who observed N had no effect on the ADF concentration of bahiagrass and other warm-season range grasses. Harvest date had a linear affect ($P < 0.01$) on ADF, with concentrations increasing with each advancing maturity. The findings of Johnson et al. (2001) also differ in regard to the effect of maturity on ADF. Johnson et al. (2001) observed a quadratic response of ADF to advancing maturity.

In situ OM digestibilities

In situ OM digestibilities were determined for wks 5, 7, and 9. A maturity × N interaction ($P = 0.03$) was observed for the A fraction. This interaction was the result of the A fraction decreasing with N provision at 5 wk of maturity and then increasing with N provision at 9 wk maturity. However, the biological significance of this observation is likely small with the values ranging from 16 to 18%. Nitrogen fertilization had no effect ($P = 0.93$) on fraction appearing in the A pool, while advancing maturity had a tendency ($P = 0.08$) to linearly increase the A fraction (Table 2). Advancing maturity from wks resulted in an increase of fraction A from 16.9 to 17.6%. The B fraction was linearly reduced ($P < 0.01$) with advancing maturity. The C fraction, which is unavailable to rumen microbes, was linearly increased ($P < 0.01$) with advancing maturity. Similar to the A fraction, N fertilization had little effect on fractions B and C ($P = 0.72$ and $P = 0.56$, respectively). This is consistent with the finding of Cuomo and Anderson (1996), they observed that N fertilization did not affect in situ digestibility of warm-season grasses. At a fixed passage rate of 3% per h, the calculated extent of OM degradation was 58.6, 54.9, and 53.4% for maturities 5, 7, and 9, respectively (linear, $P < 0.01$); Table 3. Similarly, Ruelke and Prine (1971) reported an average IVOMD of 54% for bahiagrass. In addition to the depressing effect of maturity on OM digestibility, temperature has been reported to depress the digestibility of bahiagrass (Henderson and Robinson 1982b). Henderson and Robinson (1982b) observed that the digestibility of bahiagrass decreased by 12.6% when temperature increased from 26 to 35 °C.

Table 2. Effect of N fertilization and maturity on OM fractions in bahiagrass

N Fertilization Level[a]	Harvest Date	Fraction A	Fraction B	Fraction C
		%	%	%
0	June 18	17.8	57.9	24.3
45	June 18	17.0	58.1	24.9
90	June 18	16.9	58.2	24.8
135	June 18	16.0	58.4	25.6
0	July 2	17.9	54.0	28.1
45	July 2	17.9	54.7	27.4
90	July 2	16.7	53.7	29.6
135	July 2	18.1	52.2	29.6
0	July 16	16.5	52.8	30.8
45	July 16	17.8	50.7	31.5
90	July 16	18.1	51.6	30.3
135	July 16	18.1	51.7	30.2
SEM		0.69	0.98	0.74
Maturity		0.10	<0.01	<0.01
Nitrogen		0.93	0.72	0.56
Maturity × N		0.03	0.31	0.18
Linear Maturity		0.08	<0.01	<0.01
Linear N		0.83	0.27	0.15
Quadratic N		0.98	0.98	0.97

[a]kg N/ha

Table 3. Effect of maturity on Estimated OM Digestibility and Rate of Degradation of the B Fraction

Harvest Date	OM digestibility	B fraction rate of degradation
	%	%/hr
June 18	58.6	5.3
July 2	54.9	4.9
July 16	53.4	4.7
SEM	0.73	0.22
Maturity	<0.01	0.19
Linear Maturity	<0.01	0.06

CHAPTER IV

CONCLUSIONS

Despite the drought conditions during this trail, it is clear that N fertilization has the potential to improve the nutritive value of bahiagrass and help offset the diminishing quality that comes with advancing maturity. As a result of these drought conditions the true response to increasing levels of N fertilization were not realized due to available water limiting growth. As such, we were not able to determine the level at which N fertilization becomes detrimental to forage production. Increasing N fertilization, up to a point, allows for improvement in both forage yield and CP content. Generally, these two factors are the most influential in selecting a forage for consumption by livestock. While N fertilization does have the potential to improve CP and forage yield, in this study, N was not observed to have any significant effect on the in situ degradability of OM. Further work is needed to evaluate various means of increasing the supply of N in bahiagrass and determine the impact of management of bahiagrass on utilization by ruminants.

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CONTACT INFORMATION

Name:	Nicole Marion Kenney
Professional Address:	c/o Dr. Tryon Wickersham Department of Animal Science TAMU 2471 Texas A&M University College Station, TX 77843
Email Address:	nmkenney@hotmail.com
Education:	B.A., Animal Science, Texas A&M University, May 2010 Undergraduate Research Scholar